

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
ZUMSTEIN & KLINGSEISEN
Bräuhausstr. 4
D-80331 München
GERMANY

PCT/ISA/220 (July 1998)

22 07 1999

T.

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

<p>Applicant's or agent's file reference Thaumatin-2</p> <p>International application No. PCT/EP 99/02243</p>	<p>Date of mailing (day/month/year) 17/11/1999</p> <p>FOR FURTHER ACTION See paragraphs 1 and 4 below</p> <p>International filing date (day/month/year) 01/04/1999</p>
<p>Applicant URQUIMA S.A.</p>	

1. The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for International publication.

Within 19 months from the priority date, a demand for International preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

<p>Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016</p>	<p>Authorized officer Mireille Claudepierre</p>
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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference Thaumatin-2	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 99/ 02243	International filing date (day/month/year) 01/04/1999	(Earliest) Priority Date (day/month/year) 02/04/1998
Applicant URQUIMA S.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02243

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/80	C12N15/62	C12N15/57	C12N15/56	C12N15/53
C12N15/29	C12N9/06	C12N1/15	C12Q1/68	//(C12N1/15, C12R1:665)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>MORALEJO F.J. ET AL.: "Thaumatin ✓ production in Aspergillus awamori by use of expression cassettes with strong fungal promoters and high gene dosage" APPL. ENVIRON. MICROBIOL., vol. 65, no. 3, March 1999 (1999-03), pages 1168-1174, XP002121580 the whole document</p> <p style="text-align: center;">-/-</p>	1-34

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

5 November 1999

Date of mailing of the International search report

17/11/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
 Fax (+31-70) 340-3018

Authorized officer

Galli, I

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02243

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CARDOZA R.E. ET AL.: "Characterization and nitrogen-source regulation at the transcriptional level of the gdhA gene of <i>Aspergillus awamori</i> encoding and NADP-dependent glutamate dehydrogenase." CURRENT GENETICS, vol. 34, no. 1, July 1998 (1998-07), pages 50-59, XP002121581 the whole document -& DATABASE GENBANK 'Online! Accession No. Y15784, 7 January 1999 (1999-01-07) CARDOZA R.E. ET AL.: "A. awamori gdhA gene" XP002121631 ✓ the whole document	1-15
P, X	WO 98 39459 A (DIEZ GARCIA BRUNO ;MORENO ✓ VALLE MIGUEL ANGEL (ES); SALTO MALDONADO) 11 September 1998 (1998-09-11) abstract seq. ID 1 claims 1-42	1,6
X	HAWKINS A.R.: "Nucleotide sequence and regulation of expression of the <i>Aspergillus nidulans</i> gdhA gene encoding NADP dependent glutamate dehydrogenase" MOL. GEN. GENET., vol. 218, 1989, pages 105-111, XP002121582 the whole document	1,6,35
A		2-5, 7-34
X	KINNAIRD J.H. & FINCHAM J.R.S.: "The ✓ complete nucleotide sequence of the <i>Neurospora crassa</i> am (NADP-specific glutamate dehydrogenase) gene" GENE, vol. 26, 1983, pages 253-260, XP002121583 the whole document	1,6
A	EP 0 684 312 A (URQUIMA SA) 29 November 1995 (1995-11-29) the whole document	1-34
A	GOUKA R.J. ET AL.: "Efficient production ✓ of secreted proteins by <i>Aspergillus</i> : progress, limitations and prospects" APPL. MICROBIOL. BIOTECHNOL., vol. 47, no. 1, January 1997 (1997-01), pages 1-11, XP002121584 the whole document	1-34
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 99/02243

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GOUKA R.J. ET AL.: "Analysis of heterologous protein production in defined recombinant <i>Aspergillus awamori</i> strains" APPL. ENVIRON. MICROBIOL., vol. 62, no. 6, June 1996 (1996-06), pages 1951-1957, XP002121585 the whole document	1-34
A	WO 96 36718 A (GENENCOR INT) 21 November 1996 (1996-11-21) examples 1-12 claims 1-18	1-34
A	EP 0 249 350 A (PANLABS INC) 16 December 1987 (1987-12-16) the whole document	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/02243

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9839459 ✓ A	11-09-1998 ✓	ES	2127697 A	16-04-1999
		AU	6101198 A	22-09-1998
		CA	2253250 A	11-09-1998
		CN	1219200 A	09-06-1999
		CZ	9803535 A	17-02-1999
		LT	98152 A,B	25-06-1999
		LV	12264 A	20-04-1999
		PL	329722 A	12-04-1999
		ZA	9801896 A	14-09-1998
EP 0684312 ✓ A	29-11-1995 ✓	ES	2080689 A	01-02-1996
		CA	2147541 A	22-10-1995
		FI	951868 A	22-10-1995
		JP	8047396 A	20-02-1996
		US	5932438 A	03-08-1999
WO 9636718 ✓ A	21-11-1996 ✓	CA	2221271 A	21-11-1996
		EP	0826054 A	04-03-1998
EP 0249350 ✓ A	16-12-1987 ✓	DK	288087 A	07-12-1987
		FI	872102 A	07-12-1987
		JP	63044890 A	25-02-1988

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ZUMSTEIN & KLINGSEISEN
Bräuhausstr. 4
D-80331 München
ALLEMAGNE

EINGEGANGEN

04. Juli 2000

T.

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

03.07.00

Applicant's or agent's file reference
Thaumatin-2

IMPORTANT NOTIFICATION

International application No.
PCT/EP99/02243

International filing date (day/month/year)
01/04/1999

Priority date (day/month/year)
02/04/1998

Applicant
URQUIMA S.A.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Vullo, C

Tel. +49 89 2399-8061



W 9/647543

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 October 1999 (14.10.1999)

PCT

(10) International Publication Number
WO 99/51756 A3

(51) International Patent Classification⁶: **C12N 15/80**, 15/62, 15/57, 15/56, 15/53, 15/29, 9/06, 1/15, C12Q 1/68 // (C12N 1/15, C12R 1:665)

CARDOZA SILVA, Rosa Elena [MX/ES]; Calle José Bergamin, 5, 3º-C, E-24195 Villaobispo de las Regueras (ES).

(21) International Application Number: PCT/EP99/02243

(74) Agents: ZUMSTEIN, F. et al.; Bräuhausstrasse 4, D-80331 Munich (DE).

(22) International Filing Date: 1 April 1999 (01.04.1999)

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(25) Filing Language: English

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(26) Publication Language: English

Published:

— With international search report.

(30) Priority Data:
P 9800699 2 April 1998 (02.04.1998) ES

(88) Date of publication of the international search report: 18 January 2001

(71) Applicant (*for all designated States except US*): URQUIMA, S.A. [ES/ES]; Degà Bahí, 67, E-08026 Barcelona (ES).

(15) Information about Correction:

Previous Correction:

see PCT Gazette No. 07/2000 of 17 February 2000, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 99/51756 A3

(54) Title: PROMOTER AND CONSTRUCTIONS FOR EXPRESSION OF RECOMBINANT PROTEINS IN FILAMENTOUS FUNGI

(57) Abstract: The invention relates to novel promoters of the glutamate dehydrogenase (gdh) genes from *Aspergillus awamori* and related aspergilli as well as new DNA sequences encoding glutamate dehydrogenases from *Aspergillus awamori*. The invention also relates to the use of the promoters of the gdh genes from fungus of the genus *Aspergillus* for the expression of recombinant proteins in filamentous fungi.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/02243

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/80	C12N15/62	C12N15/57	C12N15/56	C12N15/53
C12N15/29	C12N9/06	C12N1/15	C1201/68	//(C12N1/15, C12R1:665)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	MORALEJO F.J. ET AL.: "Thaumatin production in Aspergillus awamori by use of expression cassettes with strong fungal promoters and high gene dosage" APPL. ENVIRON. MICROBIOL., vol. 65, no. 3, March 1999 (1999-03), pages 1168-1174, XP002121580 the whole document --- -/-	1-34

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

' Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

5 November 1999

17/11/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

Authorized officer

Galli, I

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/02243

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
P, X	CARDOZA R.E. ET AL.: "Characterization and nitrogen-source regulation at the transcriptional level of the gdhA gene of <i>Aspergillus awamori</i> encoding and NADP-dependent glutamate dehydrogenase." CURRENT GENETICS, vol. 34, no. 1, July 1998 (1998-07), pages 50-59, XP002121581 the whole document & DATABASE GENBANK 'Online! Accession No. Y15784, 7 January 1999 (1999-01-07) CARDOZA R.E. ET AL.: "A. awamori gdhA gene" XP002121631 the whole document ---	1-15
P, X	WO 98 39459 A (DIEZ GARCIA BRUNO ;MORENO VALLE MIGUEL ANGEL (ES); SALTO MALDONADO) 11 September 1998 (1998-09-11) abstract seq. ID 1 claims 1-42 ---	1, 6
X	HAWKINS A.R.: "Nucleotide sequence and regulation of expression of the <i>Aspergillus nidulans</i> gdhA gene encoding NADP dependent glutamate dehydrogenase" MOL. GEN. GENET., vol. 218, 1989, pages 105-111, XP002121582 the whole document ---	1, 6, 35
A	---	2-5, 7-34
X	KINNAIRD J.H. & FINCHAM J.R.S.: "The complete nucleotide sequence of the <i>Neurospora crassa</i> am (NADP-specific glutamate dehydrogenase) gene" GENE, vol. 26, 1983, pages 253-260, XP002121583 the whole document ---	1, 6
A	EP 0 684 312 A (URQUIMA SA) 29 November 1995 (1995-11-29) the whole document ---	1-34
A	GOUKA R.J. ET AL.: "Efficient production of secreted proteins by <i>Aspergillus</i> : progress, limitations and prospects" APPL. MICROBIOL. BIOTECHNOL., vol. 47, no. 1, January 1997 (1997-01), pages 1-11, XP002121584 the whole document ---	1-34
	-/-	

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 99/02243

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 96 36718 A (GENENCOR INT) 21 November 1996 (1996-11-21) examples 1-12 claims 1-18 ---	1-34
A	EP 0 249 350 A (PANLABS INC) 16 December 1987 (1987-12-16) the whole document -----	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

Hui/EP 99/02243

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9839459	A	11-09-1998	ES	2127697 A	16-04-1999
			AU	6101198 A	22-09-1998
			CA	2253250 A	11-09-1998
			CN	1219200 A	09-06-1999
			CZ	9803535 A	17-02-1999
			LT	98152 A, B	25-06-1999
			LV	12264 A	20-04-1999
			PL	329722 A	12-04-1999
			ZA	9801896 A	14-09-1998
EP 0684312	A	29-11-1995	ES	2080689 A	01-02-1996
			CA	2147541 A	22-10-1995
			FI	951868 A	22-10-1995
			JP	8047396 A	20-02-1996
			US	5932438 A	03-08-1999
WO 9636718	A	21-11-1996	CA	2221271 A	21-11-1996
			EP	0826054 A	04-03-1998
EP 0249350	A	16-12-1987	DK	288087 A	07-12-1987
			FI	872102 A	07-12-1987
			JP	63044890 A	25-02-1988

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 November 1999 (24.11.99)	
International application No. PCT/EP99/02243	Applicant's or agent's file reference Thaumatin-2
International filing date (day/month/year) 01 April 1999 (01.04.99)	Priority date (day/month/year) 02 April 1998 (02.04.98)
Applicant SISNIEGA BARROSO, Heidi et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

12 October 1999 (12.10.99)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

097647548

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) Thaumatin-2

Box No. I TITLE OF INVENTION

Promoter and constructions for expression of recombinant proteins in filamentous fungi

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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 This person is also inventor.

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Teleprinter No.

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This person is applicant all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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 Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

 agent common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

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State (that is, country) of residence:
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State (that is, country) of residence:
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This person is applicant all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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State (that is, country) of residence:
Spain

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State (that is, country) of residence:
Spain

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

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State (that is, country) of residence:
Spain

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

Rosa Elena Cardoza Silva
C/ José Bergamin, 5, 3^o-C
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SPAIN

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applicant only
 applicant and inventor
 inventor only (If this check-box is marked, do not fill in below.)

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State (that is, country) of residence:
Spain

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

- AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

<input checked="" type="checkbox"/> AL Albania	<input checked="" type="checkbox"/> LS Lesotho
<input checked="" type="checkbox"/> AM Armenia	<input checked="" type="checkbox"/> LT Lithuania
<input checked="" type="checkbox"/> AT Austria	<input checked="" type="checkbox"/> LU Luxembourg
<input checked="" type="checkbox"/> AU Australia	<input checked="" type="checkbox"/> LV Latvia
<input checked="" type="checkbox"/> AZ Azerbaijan	<input checked="" type="checkbox"/> MD Republic of Moldova
<input checked="" type="checkbox"/> BA Bosnia and Herzegovina	<input checked="" type="checkbox"/> MG Madagascar
<input checked="" type="checkbox"/> BB Barbados	<input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia
<input checked="" type="checkbox"/> BG Bulgaria	<input checked="" type="checkbox"/> MN Mongolia
<input checked="" type="checkbox"/> BR Brazil	<input checked="" type="checkbox"/> MW Malawi
<input checked="" type="checkbox"/> BY Belarus	<input checked="" type="checkbox"/> MX Mexico
<input checked="" type="checkbox"/> CA Canada	<input checked="" type="checkbox"/> NO Norway
<input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein	<input checked="" type="checkbox"/> NZ New Zealand
<input checked="" type="checkbox"/> CN China	<input checked="" type="checkbox"/> PL Poland
<input checked="" type="checkbox"/> CU Cuba	<input checked="" type="checkbox"/> PT Portugal
<input checked="" type="checkbox"/> CZ Czech Republic	<input checked="" type="checkbox"/> RO Romania
<input checked="" type="checkbox"/> DE Germany	<input checked="" type="checkbox"/> RU Russian Federation
<input checked="" type="checkbox"/> DK Denmark	<input checked="" type="checkbox"/> SD Sudan
<input checked="" type="checkbox"/> EE Estonia	<input checked="" type="checkbox"/> SE Sweden
<input checked="" type="checkbox"/> ES Spain	<input checked="" type="checkbox"/> SG Singapore
<input checked="" type="checkbox"/> FI Finland	<input checked="" type="checkbox"/> SI Slovenia
<input checked="" type="checkbox"/> GB United Kingdom	<input checked="" type="checkbox"/> SK Slovakia
<input checked="" type="checkbox"/> GD Grenada	<input checked="" type="checkbox"/> SL Sierra Leone
<input checked="" type="checkbox"/> GE Georgia	<input checked="" type="checkbox"/> TJ Tajikistan
<input checked="" type="checkbox"/> GH Ghana	<input checked="" type="checkbox"/> TM Turkmenistan
<input checked="" type="checkbox"/> GM Gambia	<input checked="" type="checkbox"/> TR Turkey
<input checked="" type="checkbox"/> HR Croatia	<input checked="" type="checkbox"/> TT Trinidad and Tobago
<input checked="" type="checkbox"/> HU Hungary	<input checked="" type="checkbox"/> UA Ukraine
<input checked="" type="checkbox"/> ID Indonesia	<input checked="" type="checkbox"/> UG Uganda
<input checked="" type="checkbox"/> IL Israel	<input checked="" type="checkbox"/> US United States of America
<input checked="" type="checkbox"/> IN India	<input checked="" type="checkbox"/> UZ Uzbekistan
<input checked="" type="checkbox"/> IS Iceland	<input checked="" type="checkbox"/> VN Viet Nam
<input checked="" type="checkbox"/> JP Japan	<input checked="" type="checkbox"/> YU Yugoslavia
<input checked="" type="checkbox"/> KE Kenya	<input checked="" type="checkbox"/> ZW Zimbabwe
<input checked="" type="checkbox"/> KG Kyrgyzstan	
<input checked="" type="checkbox"/> KP Democratic People's Republic of Korea	
<input checked="" type="checkbox"/> KR Republic of Korea	
<input checked="" type="checkbox"/> KZ Kazakhstan	
<input checked="" type="checkbox"/> LC Saint Lucia	
<input checked="" type="checkbox"/> LK Sri Lanka	
<input checked="" type="checkbox"/> LR Liberia	

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- AE United Arab Emirates
- ZA South Africa
-

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) 02.04.98 April 2, 1998	98 00699	Spain		
item (2)				
item (3)				

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (*only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office*) identified above as item(s):

* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA)
(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA /

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	5
description (excluding sequence listing part)	:	42
claims	:	5
abstract	:	1
drawings	:	18
sequence listing part of description	:	5
Total number of sheets	:	76

This international application is accompanied by the item(s) marked below:

1. fee calculation sheet
2. separate signed power of attorney (10x)
3. copy of general power of attorney; reference number, if any:
4. statement explaining lack of signature
5. priority document(s) identified in Box No. VI as item(s):
6. translation of international application into (language):
7. separate indications concerning deposited microorganism or other biological material
8. nucleotide and/or amino acid sequence listing in computer readable form
9. other (specify): receipt of microorganism deposit issued by the depositary institution (5 sheets)

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Munich, 1 April 1999

Dr. F. Zumstein

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	<input type="checkbox"/> received: <input type="checkbox"/> not received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy
by the International Bureau:

See Notes to the request form

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Thaumatin-2	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP99/02243	International filing date (day/month/year) 01/04/1999	Priority date (day/month/year) 02/04/1998
International Patent Classification (IPC) or national classification and IPC C12N15/80		
Applicant URQUIMA S.A.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 9 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input checked="" type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 12/10/1999	Date of completion of this report 03.07.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bretherick, J Telephone No. +49 89 2399 8415



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/02243

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,5-48 as originally filed

2,2a,3,4 as received on 05/06/2000 with letter of 05/06/2000

Claims, No.:

1-35 as received on 05/06/2000 with letter of 05/06/2000

Drawings, sheets:

1/18-18/18 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/02243

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-15,17-35
	No:	Claims 16
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-35
Industrial applicability (IA)	Yes:	Claims 1-35
	No:	Claims

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/02243

1. This Opinion has been written on the assumption that the current priority rights in respect of the entire claimed subject-matter hold. Were this not the case, then the disclosures: Moralejo et al. (1999) Applied and Environmental Microbiology Vol. 65 pp. 1168-1174; Cardoza et al. (1998) Current Genetics Vol. 34, pp. 50-59; WO 98/39459 pub. date 11/09/1998, filing date 5/03/1998, priority 05/03/97 P 9700482 (ES) would be relevant art under Art. 33 PCT. WO98/39459 is also cited under R. 70.10 PCT (**Re. Part VI**).
2. Re. Part VIII, Art. 6 PCT:
 - a. The designations of promoters as being of various fungal origins does not describe the entities **per se** in a sufficient manner for them to be uniquely identifiable. The same applies with respect to the term "recombinant" when applied to proteins. In the case of claim 16 the use claim is not distinguishable from studies carried out in *Aspergillus* on the expression of the associated enzyme **per se**. The claim thus lacks novelty under Art. 33(1)(2) PCT.
 - c. The same applies with respect to sequences which are further defined as not being from **Aspergillus nidulans**. The use of a disclaimer is reserved, normally acceptably applying in those instances where the entity cannot be described in terms of **positive** features and where an accidental disclosure must be excluded from an otherwise novel and inventive subject-matter. In employing a disclaimer in this case, the claim remains unclear, since the precise differences existing between the subject-matter claimed and that disclaimed are not apparent. This has consequences for the acknowledgement of an inventive step under Art. 33 PCT.
 - d. With regard to the plasmid in claim 26, described by the designation pTHIX, this would appear to be clear without citing the prior art where it is first disclosed. Reference to the citation EP 684312 should therefore be deleted.
3. Re. Part V; Art. 33 PCT:
 - a. The claims 1, 3-6, 8-11, 13-15 and 27-28 are not distinguished in terms of true technical features over Hawkins et al. (1989) MOL. GEN. GENET. Vol. 218, pp.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/02243

105-111. This discloses the nucleotide sequence of the entire **A. nidulans gdhA** gene encoding NADPH dependent glutamate dehydrogenase and its promoter (Abstract, Fig. 1, legend). An acknowledgement of an inventive step under Art. 33(1)(3) PCT is therefore not possible. In the absence of prior art prejudice, the skilled person would furthermore be induced to search for genes of this type in other species of filamentous fungi using existing prior art knowledge.

b. Fincham, (1983) Gene Vol. 26 pp. 253-260; Gouka et al. (1997) Appl. Microbiol. Biotechnol. Vol. 47, pp. 1-11, EP 0 684 312 A2. Gouka et al. is a review article listing a number of promoters useable in recombinant protein expression in *Aspergillus* spp. The remaining claimed subject-matter is considered to be an obvious alternative in the light of the above. Applicant has argued in terms of extrapolation of the effectiveness of a particular promoter sequence in the expression of (heterologous) proteins in filamentous fungi. The generalisations however present in the claims extrapolate to such an extent that an inventive step on the basis of a surprising effect (i.e. increased yield) cannot be acknowledged, since promoters of the same gene in different species are now claimed in this context without proof of the effect being attributable also in the generic context.

528 Rec'd PCT/PTO 02 OCT 2000

European patent EP 684312 describes a process for preparing recombinant thaumatin in filamentous fungi. One problem of this process is that the yields obtained are low in comparison with those needed for industrial production of thaumatins.

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It is known in the art that yields of recombinant proteins can be improved when the recombinant protein of interest is expressed as a fusion with another protein, and when expression of this cassette is driven by a strong fungal promoter. This other protein, named "carrier protein", is usually a highly expressed protein of fungal origin. Up to now, the most frequently used expression system involves the glucoamylase promoter and gene from Aspergillus awamori as the promoter and the carrier protein, respectively (P.P. Ward et al., Biotechnology 1995, vol. 13, pp. 498-502). However, in some cases the use of this expression system does not lead to high levels of the desired recombinant protein. One of these specially problematic cases is the expression of recombinant thaumatin in filamentous fungi.

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Insert page 2a ←

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In view of the above, it is clear that there is the need to provide new and more efficient expression systems that allow the production of higher concentrations of those proteins that are difficult to express in filamentous fungi, such as thaumatins. This goal is achieved with the new promoter and DNA constructions provided in the present invention, as explained below.

DESCRIPTION OF THE INVENTION

30

The present invention provides a new expression system that makes use of the promoter from the glutamate dehydrogenase (gdh) gene from filamentous fungi of the genus Aspergillus, particularly from Aspergillus awamori.

35

One of the objects of the present invention is a new promoter for the expression of recombinant proteins in

MOE-ME-O

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Insertion for page 2

Gene (1983) , vol. 26, pp. 253-260 discloses the complete nucleotide sequence of the Neurospora crassa NADP-specific glutamate dehydrogenase gene.

Appl. Microbiol. Biotechnol. (1997), Vol. 47, pp 1-11 discloses the efficient production of secreted proteins by Aspergillus. Particular focus is laid on the gene fusion strategies.

EP 0 684 312 A2 relates to a preparation process of a natural protein sweetener, thaumatin. Said document discloses a new nucleotide sequence encoding thaumatin with optimised codon usage for expression in filamentous fungi.

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filamentous fungi that comprises a nucleotide sequence - or
a complementary strand thereof - selected from the group
consisting of: (a) the nucleotide sequence numbered 1-740 in
the enclosed SEQ ID No. 1; and (b) a nucleotide sequence that hy-
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bridizes under stringent conditions to that defined in (a) with the
proviso that the sequence is not the promoter of the gdh gene from
Aspergillus nidulans. Particularly preferred is the promoter
comprising the sequence defined in (a), i.e. the nucleotide
sequence numbered as 1-740 in SEQ ID No. 1, which
10 corresponds to the gdhA promoter of the glutamate
dehydrogenase A gene from Aspergillus awamori.

Although glutamate dehydrogenase A disclosed herein is the
first glutamate dehydrogenase identified and described in
15 the filamentous fungus Aspergillus awamori, there may exist
other glutamate dehydrogenases in Aspergillus awamori. The
novel nucleotide sequence of the Aspergillus awamori gdhA
promoter and/or gene shown in SEQ ID No. 1 or a portion
thereof can be used as a probe for the identification and
20 isolation of other homologous promoters/genes of glutamate
dehydrogenases in Aspergillus awamori as well as in other
organisms, preferably in filamentous fungi, more preferably
in fungi of the genus Aspergillus, still more preferably in
Aspergillus awamori and Aspergillus niger, and specially in
25 Aspergillus awamori, following the teachings of the present
invention. Consequently, the present invention is not
limited to the specific gdhA promoter from Aspergillus
awamori disclosed herein but also relates to the promoter of
any glutamate dehydrogenase gene from a fungus of the genus
30 Aspergillus with the proviso that it is not from Aspergillus
nidulans. Examples of said Aspergilli include Aspergillus
awamori, Aspergillus niger, Aspergillus oryzae and
Aspergillus sojae. In a preferred embodiment, the invention
relates to a promoter of a glutamate dehydrogenase gene from
35 Aspergillus awamori or Aspergillus niger. In a more
preferred embodiment, the invention relates to a promoter of
a glutamate dehydrogenase gene from Aspergillus awamori. The

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use of the novel nucleotide sequence shown in SEQ ID No. 1 or a portion thereof as probe is also a object of the present invention. The term "a portion thereof" denotes any part of the nucleotide sequence of SEQ ID No.1 that is 5 functional as a probe.

Another object of the present invention is a new DNA sequence, purified and isolated, that encodes a glutamate dehydrogenase protein and that comprises a nucleotide sequence - or a complementary strand thereof - selected from the group consisting of: (a) the nucleotide sequence numbered 741-2245 in the enclosed SEQ ID No. 1; and (b) 10 a nucleotide sequence that hybridizes under stringent conditions to that defined in (a) with the proviso that the sequence is not the gdh gene from Aspergillus nidulans. In a preferred embodiment, the nucleotide sequence encoding a glutamate dehydrogenase is the sequence defined in (a), i.e. the nucleotide sequence numbered as 741-2245 in SEQ ID No. 1. 15 The present invention is not limited, however, to the specific gdhA gene from Aspergillus awamori disclosed herein but also relates to any glutamate dehydrogenase gene from a fungus of the genus Aspergillus with the proviso that it is not from Aspergillus nidulans. In a preferred embodiment, the invention relates to the DNA sequences encoding 20 glutamate dehydrogenase from Aspergillus awamori or Aspergillus niger. In a more preferred embodiment, the invention relates to the DNA sequences encoding glutamate dehydrogenase from Aspergillus awamori. 25

Another object of the invention are the novel proteins encoded by any of the DNA sequences defined above. In a preferred embodiment, this protein has the amino acid sequence shown in the enclosed SEQ ID No. 2. But are also included in the present invention any glutamate 30 dehydrogenase from a fungus of the genus Aspergillus with the proviso that it is not from Aspergillus nidulans, more preferably a glutamate dehydrogenase from Aspergillus awamori or Aspergillus niger, and still more preferably a 35 glutamate dehydrogenase from Aspergillus awamori.

09/647543

05-06-2000

PCT/EP 99/02243

528 Rec'd PCT/PTO EP 009902243
02 OCT 2000

5 June 2000
90/eg/j14

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CLAIMS

1. A promoter for the expression of recombinant proteins in filamentous fungi that comprises a nucleotide sequence - or
5 a complementary strand thereof - selected from the group consisting of: (a) the nucleotide sequence numbered 1-740 in the enclosed SEQ ID No. 1; and a nucleotide sequence that hybridizes under stringent conditions to that defined in (a), with the proviso, that the sequence is not the promoter
10 of the gdh gene from Aspergillus nidulans.

2. A promoter according to claim 1 which has the sequence of nucleotides numbered 1-740 in SEQ ID No. 1 or its complementary strand.

15 3. Isolated promoter of a glutamate dehydrogenase gene from a fungus of the genus Aspergillus with the proviso, that the sequence is not the promoter of the gdh gene from Aspergillus nidulans.

20 4. Isolated promoter according to claim 3 wherein the fungus is Aspergillus awamori or Aspergillus niger.

25 5. Isolated promoter according to claim 4 wherein the fungus is Aspergillus awamori.

30 6. A purified and isolated DNA sequence that encodes a glutamate dehydrogenase protein and that comprises a nucleotide sequence - or a complementary strand thereof - selected from the group consisting of: (a) the nucleotide sequence numbered 741-2245 in the enclosed SEQ ID No. 1; and (b) a nucleotide sequence that hybridizes under stringent conditions to that defined in (a), with the

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proviso, that the sequence is not the gdh gene from Aspergillus nidulans.

7. A DNA sequence according to claim 6 which has the
5 sequence of nucleotides numbered 741-2242 in SEQ ID No. 1,
or its complementary strand.

8. An isolated DNA sequence encoding a glutamate
dehydrogenase from a fungus of the genus Aspergillus, with
10 the proviso, that the sequence is not the gdh gene from
Aspergillus nidulans.

9. An isolated DNA sequence according to claim 8 wherein the
fungus is Aspergillus awamori or Aspergillus niger.

15 10. An isolated DNA sequence according to claim 9 wherein
the fungus is Aspergillus awamori.

11. The protein encoded by any of the DNA sequences
20 according to claim 6.

12. The protein which has the amino acid sequence in SEQ ID
No. 2.

25 13. An isolated glutamate dehydrogenase from a fungus of
the genus Aspergillus with the proviso, that the glutamate
dehydrogenase is not the glutamate dehydrogenase from
Aspergillus nidulans.

30 14. An isolated glutamate dehydrogenase according to claim
13, wherein the fungus is Aspergillus awamori or Aspergillus
niger.

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15. An isolated glutamate dehydrogenase according to claim
14, wherein the fungus is Aspergillus awamori.

16. Use of a promoter from a glutamate dehydrogenase gene
5 from a fungus of the genus Aspergillus for the expression of
recombinant proteins in filamentous fungi.

17. Use according to claim 16, wherein the promoter is a
promoter according to any one of claims 1 to 5.

10 18. A DNA construction that comprises: a) a promoter from a
glutamate dehydrogenase gene from a fungus of the genus
Aspergillus; b) a DNA sequence encoding a protein normally
expressed from a filamentous fungus or a portion thereof; c)
15 a DNA sequence encoding a cleavable linker peptide; and d) a
DNA sequence encoding a desired protein.

19. A DNA construction according to claim 18, wherein the
promoter under a) is a promoter according to any one of
20 claims 1 to 5.

20. A DNA construction according to claim 18, wherein the
DNA sequence under b) encodes a protein or portion thereof
selected from the group consisting of: i) glucoamylase from
25 Aspergillus awamori, Aspergillus niger, Aspergillus oryzae
or Aspergillus sojae; ii) B2 from Acremonium chrysogenum;
and iii) a glutamate dehydrogenase from a filamentous
fungus.

30 21. A DNA construction according to claim 20, wherein the
DNA sequence under b) encodes glucoamylase from Aspergillus
awamori Aspergillus niger, Aspergillus oryzae or Aspergillus
sojae or a portion thereof.

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22. A DNA construction according to claim 20, wherein the DNA sequence under b) encodes the protein B2 from Acremonium chrysogenum or a portion thereof.

5 23. A DNA construction according to claim 20, wherein the DNA sequence under b) encodes a glutamate dehydrogenase from a filamentous fungus or a portion thereof.

10 24. A DNA construction according to claim 18, wherein the DNA sequence under c) contains a KEX2 processing sequence.

25. A DNA construction according to any one of claims 18 to 24, wherein the DNA sequence under d) encodes thaumatin.

15 26. A DNA construction according to claim 25, wherein the DNA sequence under d) is the thaumatin II synthetic gene from plasmid pThIX disclosed in EP 684312.

20 27. A DNA construction comprising a gdh promoter from a fungus of the genus Aspergillus operatively linked to a DNA sequence encoding a recombinant protein.

25 28. A DNA construction according to claim 27, wherein the promoter is a promoter according to any one of claims 1 to 5.

30 29. A filamentous fungus culture capable of producing a recombinant protein which has been transformed with a plasmid containing a DNA construction according to any one of claims 18 to 28.

30. A culture according to claim 29, wherein the filamentous fungus is a fungus from the genus Aspergillus.

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31. A culture according to claim 29, wherein the filamentous fungus is selected from the group consisting of Aspergillus awamori, Aspergillus niger, Aspergillus oryzae, Aspergillus nidulans and Aspergillus sojae.

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32. A culture according to claim 29, wherein the plasmid contains a DNA construction according to any one of claims 25 or 26.

10 33. A process for producing a recombinant protein in a filamentous fungus comprising the following steps:

a) preparation of an expression plasmid containing a DNA construct according to any of claims 18 to 28;

15 b) transformation of a strain of filamentous fungus with said expression plasmid;

c) culture of the transformed strain under appropriate nutrient conditions to produce the desired protein, either intracellularly, extracellularly or in both ways simultaneously; and

20 d) depending on the case, separation and purification of the desired protein from the fermentation broth.

25 34. A process according to claim 33, wherein the recombinant protein is thaumatin and the expression plasmid contains a DNA construction according to claims 25 or 26.

30 35. Use of a DNA sequence derived from a nucleotide sequence according to claims 1 to 6 as a probe for the identification and isolation of a glutamate dehydrogenase gene and/or a promoter sequence of a glutamate dehydrogenase gene.